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The influence of hydrogen ion concentration upon the action of the amylase of Aspergillus niger

BY

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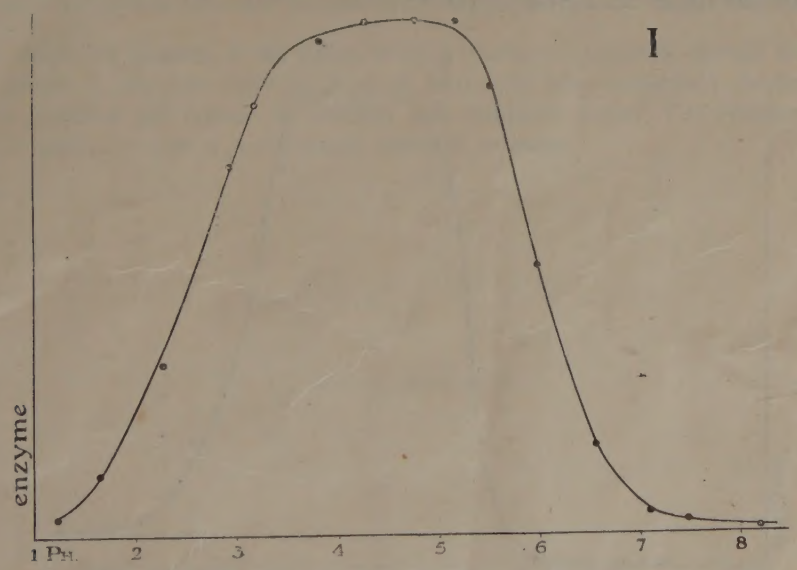
Aspergillus niger produces large quantities of amylase, part of which migrates into its nutritive surrounding. In the mean time the fungus forms acids which cause that medium to have a high hydrogen ion concentration. As this however seemed not to influence unfavourably the action of the amylase, the supposition was justified that the amylase of Aspergillus niger could not have its optimal action at the same hydrogen ion concentration as the ptyaline which works best at a nearly neutral or faintly acid reaction (4 and 5).

Therefore I made a preliminary investigation in the way as has been indicated first by SÖRENSEN (1). Buffer solutions however were made according to the methods of CLARK and LUBS (7).

Generally the same amounts of enzyme solution out of the nutritive liquid were mixed up with buffer solution and amylum



solution 0.16 %. The hydrogen ion concentration of this mixture was determined by aid of colorimetric indicators, the rate of hydrolysis of the amylum by the iodine reaction.

Results are plotted into the annexed curve (I). As can be seen there is no point of optimal action but a broad optimal zône extending from a P_H of about 3,5 till about 5,5.

Neither the concentration of the amylase, nor the composition of the nutritive liquid appeared to have influence. The same results were obtained with amylase extracted from the mycelium.

These results largely confirm the theory of MICHAËLIS who considers the enzymes as ampholytes (2 and 3). The form of the curve indeed is nearly identical to the dissociation rest curve of an amphotere electrolyte. According to his formulas

$$\varrho_a = \frac{1}{1 + \frac{K_a}{(H)}} \quad \text{and} \quad \varrho_b = \frac{1}{1 + \frac{K_b}{(OH)}}$$

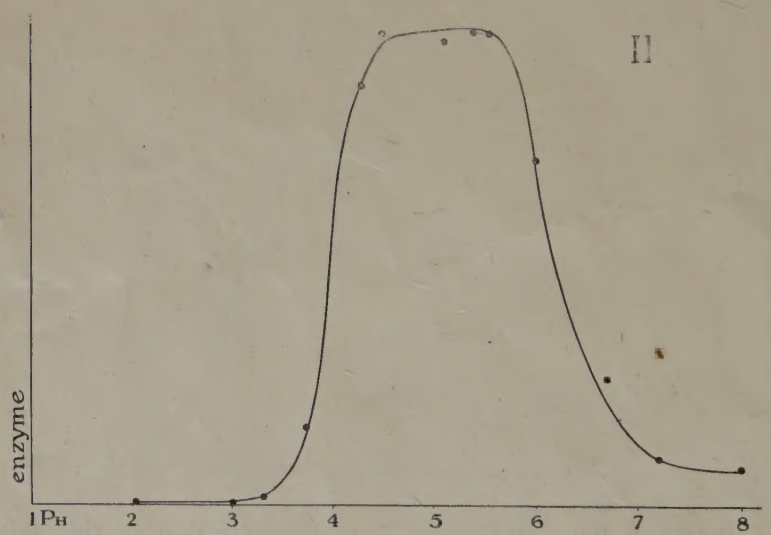
in which $\varrho = 1 - \gamma =$ dissociation rest

$\gamma =$ rate of dissociation

$K_a =$ dissociation constant of the acid

$K_b =$ dissociation constant of the base

the points on the ordinate = half of the maximum height of the curve indicate the logarithms of the dissociation constants of acid and base on the abscissa. These are to be found at about 2,26 and 6,2. So the dissociation constant of the acid would be $= 6.3 \times 10^{-7}$, that of the base $= 2.884 \times 10^{-12}$.



We may consider in the same way curve II which represents

the influence of the hydrogen ion concentration upon the amylase of malt[1]).

The dissociation constant of the acid appears to be the same as for the amylase of Aspergillus, that of the base on the contrary is bigger i.e. $= 5.76 \times 10^{-11}$. So as an acid the two amylases are equally strong, as a base that of the malt is the weakest.

Further investigations on other sorts of amylase will perhaps instruct us, if pointing out their differences in this way will be of any value.

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LITERATURE.

1. Sörensen S. P. L., Biochem. Zeitschr. Bd. 21, 1909. Enzymstudien II.

2. Michaelis, L., Biochem. Zeitschr. Band 33, 1911. Ueber die Dissoziation der amphoteren Electrolyte.

3. Michaelis L. und Davidsohn, Biochem. Zeitschr. Band 35, 1911. Die Wirkung der H. Ionen auf das Invertin.

4. Ringer, W. E. en Trigt H. van, Onderzoek. Physiol. lab. der Un. Utrecht. 5e reeks, dl. 14, 1913. Over den invloed van de reactie op de werking van ptyaline.

5. Michaelis L. und Pechstein, H., Bioch. Zeitschr. Band 59, 1914. Die Wirkungsbedingungen der Speicheldiastase.

6. Adler, L., Biochem. Zeitschr. Band 77, 1916. Ueber den Einfluss der Wasserstoffionen auf die Wirksamkeit der Malzdiastase.

7. Clark, W. M. and Lubs, H. Am., Journ. of Bact. Vol. II. 1917. The colorimetric determination of hydrogen ion concentration and its applications in bacteriology.

[1]) It might be doubted if the iodine reaction method is accurate enough to get exact results. I therefore refer to those of Adler (6) who determined the hydrolysis of amylum by means of rotation and reductive power. The numbers he obtained appear to give a curve nearly identical to mine.